RNA synthesis in salmonella typhimurium

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SUMMARY

In this thesis several aspects of RNA synthesis in two strains of *Salmonella typhimurium*, a bacterial species closely related to the well-known work-horse of molecular biology, *Escherichia coli*, are described. One of the strains shows a greatly reduced RNA synthesis under conditions causing amino acid starvation, whereas in the other amino acid deprivation does not repress RNA synthesis.

This difference can be ascribed to one gene that can be present in either one of two allelic states. If the cells carry the RC^{str} allele - the normal situation in bacteria - amino acid deprivation leads to a drastic reduction in RNA synthesis. If, however, the RC^{rel} allele is present - a situation only found in *E. coli* and *S. typhimurium* so far - amino acid starvation has no or hardly any effect on RNA synthesis. The two *S. typhimurium* strains used in this study were, as far as known, genetically identical (isogenic) except for the RC gene.

A general introduction given in Chapter I relates the purpose of this study as well as the reasons why it branched into experiments on the maturation of a specific type of RNA, low molecular weight ribosomal (5-S) RNA. Chapter II serves to acquaint the reader with the present state of knowledge on regulation of bacterial RNA synthesis, with emphasis on the regulation by amino acid availability (RC type of regulation). In this chapter the various ways in which this type of regulation manifests itself are set forth and the models proposed for its explanation are subjected to a critical discussion.

Chapter III is concerned with the properties of 5-S RNA. The available data on the genetics, primary and secondary structure, function, mode of synthesis and mechanism of maturation of this unique species of low molecular weight bacterial RNA are described.

Chapter IV gives the detailed methods used in the course of our studies.

The experimental part of this thesis starts with Chapter V, which describes a more or less technical digression made necessary by the fact that methylated albu-

min kieselguhr chromatography, routinely used for RNA analysis, under our conditions produced two peaks of what was later shown to be 5-S RNA, instead of one. We could show that this abnormal behaviour was due to denaturation of part of the 5-S RNA very likely caused by the use of an elevated temperature (35°C) and this column material.

Chapter VI is concerned with the effects of various treatments decreasing protein synthesis on overall RNA synthesis as well as the synthesis of specific types of RNA. These treatments included amino acid starvation, addition of various doses of chloramphenicol to starved and non-starved cells and addition of trimethoprim in the presence and absence of chloramphenicol.

The main conclusion that can be drawn from these experiments is the following: The presence of the RC^{rel} allele of the RC gene has only a quantitative effect on the synthesis of stable RNA species [synthesis of unstable (messenger) RNA was not studied, neither was synthesis of (stable) 6-S RNA]. Under identical conditions no qualitative differences between the RNA synthesized by either strain (which we had hoped to find) could be detected. Moreover, it appeared that synthesis of the various species of stable RNA normally present in bacteria - 5-S, 16-S, 23-S and tRNA (again excepting 6-S RNA) - or their precursors, remains coordinate under almost all conditions tested. Only in the presence of high doses of chloramphenicol, resulting in virtual absence op protein synthesis, 23-S RNA is labelled to a lesser extent than the other species of stable RNA. Arguments, but no proof, are given for the idea that this is not the result of increased breakdown of 23-S RNA under these conditions but rather of a decreased synthesis relative to the synthesis of 16-S, 5-S and tRNA.

It can also be concluded that the effects of the RC gene on RNA synthesis as a whole as well as synthesis of different types of RNA in *S. typhimurium* are very similar, if not identical, to those described for *E. coli*, under the conditions tested. This indicates that the two bacterial species have the same mechanism responsible for the effect of amino acid starvation on RNA synthesis. Moreover, the difference(s) between an RC^{str} and an RC^{rel}

strain, as shown by our experiments, can not reside in their respective RNAs and very likely neither in their respective RNA genes.

In the second experimental part of the thesis (Chapter VII) the discovery of a form of 5-S RNA in *S. typhimurium* showing properties very similar to the precursor 5-S RNA isolated from *E. coli* is described. The maturation of this precursor was studied in relation to the synthesis of the large ribosomal (50-S) subunit with which 5-S RNA is associated in exponentially growing cells. It is shown that maturation of each molecule of 5-S RNA is dependent on the synthesis of one 50-S ribosomal subunit to at least the 40-S stage. Moreover, our results strongly indicate that this maturation is a step in the assembly process of the large ribosomal subunit since no mature 5-S RNA that was not associated with a ribosomal or subribosomal particle could be detected.